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The Presence of a Myxobacterium, *Chondrococcus columnaris* (Davis) Ordal and Rucker (1944), on *Fundulus heteroclitus* (Linn.).

ROSS F. NIGRELLI
New York Zoological Society
&
S. H. HUTNER
Haskins Laboratories

(Plate I).

INTRODUCTION.

Davis (1922) recorded an organism inducing a fatal disease among certain fishes in the warm fresh waters of the Mississippi Valley. The disease is manifest as a mold-like growth on the skin. The lesion is one of simple tissue destruction in which the epidermis and the underlying layers are gradually disintegrated by the action of the organisms. The microorganism producing this disease was called *Bacillus columnaris* by Davis. He also observed the disease in smallmouth bass and common perch in the St. Lawrence River at Ogdensburg, N. Y. Nigrelli (1943) found a similar organism on catfishes (*Ameiurus platycephalus, A. nebulosus* and *Opladelus olivaris*) in the tanks of the New York Aquarium. The infection made its appearance in the spring of the year while the temperature was rising, and persisted for most of the summer.

Ordal and Rucker (1944) and Garnjobst (1945) independently discovered the true nature of this microorganism. It was placed in the "slime bacteria" (Order, Myxobacteriales). This is the first member of this interesting group to be reported as pathogenic. The life history is divided into two stages: the first, or swarm stage, is a period of active multiplication of rod-shaped motile cells; the second is a period in which they become quiescent and enclosed in cysts, forming fruiting bodies. The second part of this life cycle was noted by Ordal and Rucker in material cultured from infections in fingerling blueback salmon (*Oncorhynchus nerka*) reared in hatcheries. They also observed these fruiting bodies in material from adult fishes taken from the Columbia River. From this and other morphological observations they assigned the species to the genus *Chondrococcus*, family Myxococcaceae. Similar fruiting bodies were noted in the material found on *Fundulus heteroclitus* reported in the present paper. Garnjobst (1945), however, did not find a fruiting body stage in her material, and because of this called the organism *Cytophaga columnaris*. It is not certain whether or not these investigators are dealing with the same strain or even the same species. Ordal and Rucker (1944) reported that they found a second strain of myxobacteria from the gills of trout and salmon fingerlings which did not produce fruiting bodies.

In the present studies, we have had the opportunity to compare our organism with a strain of *Chondrococcus columnaris* very kindly sent us by Ordal and Rucker. Ordal and Rucker's strain and our strain appeared morphologically and biochemically alike.

Chrondrococcus columnaris FROM *Fundulus heteroclitus*.

During the spring of this year an epizootic occurred among killifish brought in from Long Island Sound. in the vicinity of Pelham Bay, New York City. The majority of fish were infected either with gyrodactylid trematodes or with the protozoan *Trichodina*. There was no evidence of *Chondrococcus columnaris*, an infection which is unmistakable when present. Several hundred fish were crowded into a large fresh water tank. The temperature was about 24° C. and the pH about 7.8. At the end of three days many of the fish showed a fungus-like growth, typical of this myxobacterium (Plate I). The growth was grayish with a slightly brownish cast.

Microscopical examination of fresh material showed long slender flexible rods characteristic of the Myxococcaceae. No motility was seen in these water mounts. The cells

were mostly of one type, although occasionally some of the variants described by Garnjobst were present in cultured material. Living vegetative cells measured 4 to 12 microns in length and 0.3 to 0.7 microns in width. They were gram negative. Some of the preparations made from the growth taken from the fish showed spherical brownish masses typical of the fruiting bodies reported and figured by Ordal and Rucker.

Isolation of the organism in pure culture was successful. The shake dilution method was employed, using filtered and sterilized tank water. A buffered semi-solid medium containing 0.2% Bacto-peptone and 0.25% agar was used for culturing. The cultures were kept at room temperature and good growths were obtained in about 48-96 hours. The colonies were yellowish in color, forming flat sheets with irregular outline. Examination of the cultures from time to time showed swarms of flexible rods. In older cultures, brownish fruiting bodies were also present.

Morphologically and physiologically, the *Chondrococcus* isolated from the killifish was similar to the strain sent to us by Ordal and Rucker. The organisms were not influenced by the addition of carbohydrates to the medium, nor were they able to utilize large but non-toxic amounts of lactate, malate, acetate, butyrate, succinate, pyruvate, l-aspartate, l-asparagin, l-glutinate, and dl-alanine. They grew well in a complete mixture of amino acids and also in gelatin and casein hydrolysates. Tryptone and the hydrolysates were about of equal efficacy. Yeast extract (Difco) was definitely inferior. The evidence indicates that no unknown growth factors are required. However, the essential amino acids were not identified. It is concluded from these observations that amino acids are used for energetic as well as for structural purposes.

As was pointed out above, the pathological effects result from tissue destruction. The organisms grow on the surface of the body and extend into the branchial chambers. Eventually the delicate gill tissues become involved, in which case the disease is fatal. This pathogenesis was noted by Davis (1922). Ordal and Rucker (1944) reported that myxobacteria were found in the internal organs of adult chinook and blueblack salmon, steelhead trout, squawfish, whitefish, chubs and suckers taken from the Columbia River. No infection of the internal organs was found in killifish examined. Fish showing typical external lesions were autopsied and the internal organs were found unaffected. Smears made of the spleen, kidneys, heart and intestine showed no organisms recognized as myxobacteria.

Whether the myxobacteria found in the killifish also occur in the feral state was not determined. As was mentioned above the fish were brought in from the brackish water of the Sound. No typical growths were observed on their arrival. The infection became evident several days after they had been placed in fresh water. The disease was limited in its course to killifish even though other species were present in tanks supplied by the same circulating water. The fish in these other tanks included forms known to be highly susceptible to myxobacteria (e.g., catfish). This would indicate that the pathogen is either a highly specific strain or that, like *Saprolegnia*, it is an invader which infects only fish with abrasions, or fish with other primary lesions such as those due to parasitic protozoa or trematodes. If the latter is true the organism is not a strict parasite but rather a saprophyte like the nonpathogenic members of this highly organized group. From these considerations one may predict that myxobacteria pathogenic for fish will be found to be cosmopolitan in distribution. Myxobacteria have already been reported from fish in waters of Washington, Mississippi Valley, West Virginia, northern and southern New York State.

Summary.

A myxobacterium, *Chondrococcus columnaris* (Davis), is reported for the first time from the skin and gills of the common killifish, *Fundulus heteroclitus*, caught in the vicinity of Pelham Bay, New York City. The organism was isolated and cultured in a semi-solid medium containing 0.2% Bacto-peptone and 0.25% agar. Typical swarming and fruiting bodies were encountered in fresh and cultured material. This myxobacterium resembles one of the strains reported by Ordal and Rucker (1944) in the presence of the fruiting stage in the life cycle. Other strains reported by these investigators, and a form described by Garnjobst (1945) as *Cytophaga columnaris* (Davis), lack these fruiting bodies. The evidence indicates that no unknown growth factors are required. The organism grew well in a complete mixture of amino acids, as well as in gelatin and casein hydrolysates. The essential amino acids, however, were not identified.

References.

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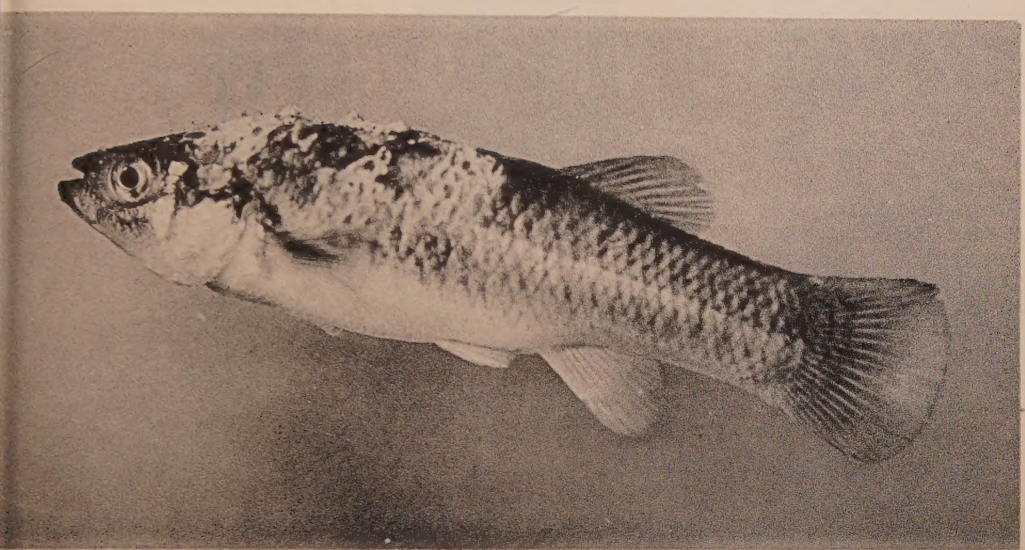
EXPLANATION OF THE PLATE.

Killifish, *Fundulus heteroclitus*, showing infection of a myxobacterium, *Chondrococcus columnaris* (Davis). About natural size. Photograph by S. C. Dunton, N. Y. Zoological Society.



THE PRESENCE OF A MYXOBACTERIUM, CHONDROCOCCUS COLUMNARIS (DAVIS) ORDAL AND RUCKER (1944), ON FUNDULUS HETEROCLITUS (LINN.).